Hypothesis

A Hypothetical Framework for Genotoxic and Proteotoxic Risk via Covalent Lipid and Nucleic Acid Adduct Formations in mRNA LNP Vaccines

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A Hypothetical Framework for Genotoxic and Proteotoxic Risk via Covalent Lipid and Nucleic Acid Adduct Formations in mRNA LNP Vaccines

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Abstract

Adductomics is the systematic study of covalent modifications to biomolecules, providing a framework for evaluating potential biological concerns in pharmaceuticals. Historical precedents, including withdrawals of medications like fialuridine and troglitazone, demonstrate that even low frequency adduct formation can lead to genotoxic or proteotoxic events. Recent Moderna funded research found that cationic and ionizable lipid nanoparticles can form covalent adducts with modified RNA nucleotides under assay conditions, with adduct levels increasing at physiological temperatures approaching those of the human body. These findings raise the hypothesis that in vivo environments--characterized by 37 °C, complex ionic composition, reactive oxygen species, dynamic zeta potential, and persistence of synthetic RNA for weeks--could amplify the frequency or diversity of these adducts. Reactive lipids could also theoretically bind to residual linearized plasmid DNA contaminants in COVID or other modified RNA and LNP based vaccines, or interact with endogenous nucleic acids, introducing pathways for covalent modifications that may alter nuclear DNA interactions, RNA, chromatin structure, proteins, and protein associations. These considerations underscore the need for proper systematic adductomic, proteomic, and genotoxicity testing of RNA lipid nanoparticle therapeutics to evaluate potential in vivo risks before continued clinical deployment.

Keywords: RNA LNP vaccines; lipid RNA adducts; genotoxic impurities (GTIs); adductomics; DNA contaminants; oxidative stress; reactive lipid species; protein adductome; vaccine safety; biomonitoring

Introduction

The rise of RNA based therapeutics and vaccines has driven extensive use of lipid nanoparticle (LNP) delivery systems, which enhance RNA stability, cellular uptake, and endosomal release [1]. Recent Moderna research indicates that certain chemical impurities within these lipids can, in low numbers, form covalent interactions with nucleic acids [2]. These findings raise concerns around the assumption that LNPs are chemically inactive and suggest potential for molecular level reactivity under physiological conditions.

Adducts are covalent modifications that occur when reactive chemical species bind to biomolecules like RNA, DNA, or proteins [3]. The field of adductomics systematically studies these modifications to map the frequency, location, and consequences of covalent binding [4]. Understanding these interactions is critical for RNA therapeutics, as adduct formation could alter nucleic acid stability, interfere with translation, or modify protein function, providing a mechanistic basis for evaluating molecular safety in novel drug platforms.

Historical precedent demonstrates that rare adduct formation can trigger toxicity [5]. Drugs like fialuridine, troglitazone, and benoxaprofen were withdrawn after reactive metabolite adducts caused severe organ damage [6]. The Moderna study provides the first direct evidence of covalent RNA lipid adducts under assay conditions that approximate clinical formulations [2], emphasizing the need to

determine whether such interactions could occur in vivo, particularly with persistent modified RNA, reactive lipids, or residual linearized plasmid DNA, and what implications these adducts may have for nucleic acid and protein integrity.

1. Adductomics and the Mapping of Covalent Modifications

The field of adductomics has emerged to systematically map these covalent modifications across entire biomolecular classes. Initial studies in the 1990s focused primarily on DNA adducts induced by reactive small molecules, with pioneering researchers like Turesky and Randerath developing sensitive mass spectrometry-based approaches to detect modifications at extremely low frequencies [6]. Over the decades, analytical methodologies expanded to include reversed-phase ion-pair high-performance liquid chromatography (HPLC), high-resolution liquid chromatography–tandem mass spectrometry (LC-MS/MS), and immunoassays [7]. These techniques allow researchers to detect DNA, RNA, and protein adducts even in minimal tissue samples, facilitating quantitative mapping of adduct type, location, and abundance. Collectively, this body of modifications constitutes the adductome--the complete set of covalent adducts present in a biological system at a given time [8].

Adducts are covalent chemical modifications that occur when reactive species bind to biomolecules, including nucleic acids, proteins, and small molecules [3]. These modifications arise through a variety of chemical mechanisms, most commonly via electrophilic attack on nucleophilic sites. Electrophilic metabolites derived from environmental chemicals, diet, pharmaceuticals, or endogenous processes including reactive carbonyls, aldehydes, epoxides, or α,β-unsaturated carbonyl compounds can react with nucleobases, amino acid residues, or small molecules to form stable covalent linkages like Schiff bases, Michael adducts, or cross-links [9,10].

Aldehydes can form Schiff bases with lysine residues in proteins, while polycyclic aromatic hydrocarbons (PAHs), nitrosamines, and aristolochic acids can generate DNA adducts at exocyclic nitrogen atoms [11]. Reactive intermediates generated through oxidative stress, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), can similarly damage nucleic acids, producing oxidized lesions like 8-oxo-7,8-dihydroguanine (8-oxoG), formamidopyrimidine derivatives, or 5-hydroxymethylcytosine [12,13]. Even low frequency adducts, present at sub-percent levels, can exert profound biological effects, potentially altering macromolecular structure, interfering with enzymatic or polymerase activity, inducing mutagenesis, or triggering immune responses, which under certain conditions, may lead to disease including organ damage, neurological diseases, autoimmune like disorders, and cancer [6,14]. The kinetics of adduct formation, chemical reactivity, and cellular persistence, alongside other factors, collectively determine the likelihood and magnitude of their biological impact [6].

Humans are continuously exposed to hazardous chemicals in the environment and diet, as well as to endogenously produced electrophiles, all of which can form covalent adducts with DNA and proteins [15]. While enzymatic repair systems exist to correct many adducts, a subset may escape repair, and these unrepaired modifications can induce mutations during cell division, initiating carcinogenesis or other pathologies [15]. DNA adducts at driver mutation sites, like in the H-ras, K-ras oncogenes, or p53 tumor suppressor gene, can initiate chemical carcinogenesis in both experimental models and human cancers associated with environmental exposures [6]. Environmental carcinogens including PAHs, nitrosamines, aflatoxins, aristolochic acids, haloalkanes, and aromatic amines form adducts preferentially at nucleophilic positions like N7 of guanine, N6 of adenine, or exocyclic oxygens [17]. Steric hinderance, chemical reactivity, and the ability of electrophiles to intercalate into DNA influence the preferential modification of these sites. Additionally, reactive intermediates may form adducts on the phosphate backbones of nucleic acids, further complicating the range of possible lesions [17].

Reactive oxygen species generated during oxidative stress or inflammation create another major class of adducts. ROS attack nucleobases or the deoxyribose backbone, producing oxidized lesions like 8-oxoG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-Gua), 8-oxo-Ade, 5-hydroxycytosine, and 5-hydroxymethylcytosine, which can miscode during replication and

transcription, contributing to mutagenesis and genomic instability [18,19]. 8-oxoG is a common type of oxidative DNA damage that can mispair with adenine during DNA replication, leading to a permanent G-to-T mutation in the DNA sequence. This G-to-T change can be passed down to future generations if it occurs in germline cells in the sperm or egg [20,21].

Lipid peroxidation of polyunsaturated fatty acids produces reactive aldehydes, like malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE), and acrolein [21]. These species react with DNA to form endocyclic or exocyclic adducts, including M1dG, 1,N2-propano-dGuo adducts, and etheno-adducts (N2,3εdG, 1,N2εdG, εdA, and εdC), and with proteins at lysine, cysteine, or histidine residues [21]. Even small, transient adducts may propagate damage across cellular networks, impairing enzymatic activity, signaling pathways, or generating neoantigens capable of eliciting immune responses, and potential for oncogenesis [21].

Mapping the adductome has crucial biological relevance. In nucleic acids, covalent modifications can stall polymerases, misdirect DNA or RNA repair pathways, or alter transcription fidelity, potentially leading to mutations, genomic instability, or chemical carcinogenesis [22]. Proteins are similarly susceptible: covalent modifications can alter enzymatic activity, disrupt signaling pathways, interfere with protein folding or interactions, and generate neoantigens [23]. Historical examples underscore the importance of adduct mapping: the nucleoside analog fialuridine incorporated into mitochondrial DNA, causing fatal hepatotoxicity; troglitazone formed DNA and protein adducts, resulting in hepatotoxicity; and benoxaprofen generated photoactivated DNA and protein adducts, leading to cytotoxicity [24]. These cases illustrate that adduct frequency alone does not predict risk; molecular context, persistence, and exposure of vulnerable systems are critical determinants [22–24].

Chemical metabolism and bioactivation play a central role in adduct formation. Many xenobiotics require enzymatic conversion by Phase I enzymes, like cytochrome P450 monooxygenases, to generate electrophilic metabolites capable of covalent binding [25]. Subsequent Phase II conjugation reactions, generally considered detoxifying, can occasionally yield reactive intermediates capable of forming adducts. Examples include O-acetylation or O-sulfation of hydroxylamines, glutathione conjugation of haloalkanes, and acyl glucuronidation of nonsteroidal anti-inflammatory drugs [26]. Endogenous reactive species generated via oxidative stress, inflammation, or lipid peroxidation similarly expand the repertoire of covalent adducts in vivo, underscoring the complex interplay between exposure, metabolism, and chemical reactivity in shaping the adductome [27].

By integrating high resolution analytical methods with an understanding of chemical reactivity, persistence, and biological context, adductomics allows researchers to systematically map the full landscape of covalent modifications [28]. This mapping provides mechanistic insight into chemical toxicity, mutagenesis, and disease etiology, enabling biomonitoring of exposures in humans, identification of potential carcinogens, and evaluation of molecular consequences of environmental, dietary, or pharmacological chemicals [29]. The adductome therefore serves both as a molecular fingerprint of exposure and as a framework for understanding the biological impact of covalent modifications across nucleic acids, proteins, and small molecules [30].

1.1. Genotoxic Impurities (GITs) and Regulatory Thresholds

The presence of reactive impurities in pharmaceutical products has long been recognized as a potential driver of DNA damage and carcinogenic risk [31]. Within the small molecule domain, this concern led to the development of internationally harmonized regulatory frameworks like ICH M7 (R1)--Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals. ICH M7 (R1) provides a science and risk-based approach for the identification, evaluation, and control of genotoxic impurities, emphasizing structural assessment, computational predictions, and confirmatory in vitro testing [33]. A central feature of this guideline is the threshold of toxicological concern (TTC), typically set at 1.5 μg/day for lifetime exposure, which is designed to minimize excess cancer risk across diverse populations. Impurities exceeding this limit must be justified with

compound specific toxicological data or controlled to levels below the TTC [33]. The current molar ratios and impurity amounts contained within the current modified RNA and LNP COVID vaccines may exceed this amount, falling outside of ICH M7 regulatory guidelines for potential presence of genotoxic substances within the drug compound.

Complementing ICH M7, the U.S. Food and Drug Administration (FDA) has issued guidance documents that align with, but also expand upon, the international standard. The FDA underscores the importance of early identification of GTIs during the IND stage, the use of validated analytical methods (LC–MS/MS, GC–MS) with high sensitivity and reproducibility, and the development of proactive impurity control strategies across the drug development lifecycle [34]. While generally supportive of the TTC based approach, the FDA notes that stricter impurity limits may be required for compounds with established carcinogenicity or structural alerts known to produce DNA adducts [34].

Traditionally, genotoxic impurities have been considered within the context of small molecule drugs, where reactive electrophiles or synthetic byproducts can covalently bind DNA [35]. However, the emerging data from LNP RNA platforms might suggest that this paradigm may warrant extension [2]. Ionizable lipid impurities capable of forming reactive aldehydes or electrophilic species could, in principle, act as GTIs if they establish covalent bonds with DNA or other nucleic acids--whether endogenous genomic DNA or exogenous plasmid DNA contaminants that have been documented in some vaccine lots [36]. ROS, generated in vivo under physiological conditions, may amplify this process by promoting lipid peroxidation and secondary electrophile formation [37]. This raises the possibility of downstream 8-oxoG lesions, single and double-strand breaks, and error-prone repair events, which mirror the canonical endpoints of genotoxic exposure.

The pharmaceutical field offers several historical precedents underscoring the regulatory significance of GTIs. Certain drugs have been withdrawn from the market or subjected to restricted use due to liabilities linked to DNA adduct formation or genotoxic degradation products. These examples illustrate the strict enforcement landscape surrounding GTIs, as well as the recognition that even low-level impurities may carry disproportionate long-term risk [38].

Applying this context to RNA LNP platforms, the unanswered question is whether reactive lipid-derived species--documented in the Moderna characterization study [2]--might exhibit genotoxic behavior once delivered in vivo, especially in the presence of co-packaged DNA fragments [36]. The absence of adductomic or genotoxicity data specific to these platforms leaves a critical gap in the safety database. If these impurities do function as GTIs, with or without the presence of modified RNA or linearized DNA plasmids, their risk cannot be presumed negligible by analogy to conventional excipients, but must instead be empirically evaluated using the same scientific and regulatory rigor applied to small molecule therapeutics.

2. The Moderna Study

LNP mRNA vaccines like the Moderna and Pfizer/BioNTech SARS-CoV-2 vaccines, rely critically on the stability and integrity of encapsulated mRNA to ensure robust protein expression [39]. The labile nature of mRNA poses a significant challenge for long term stability, particularly during storage and distribution. Traditional degradation pathways, including hydrolysis, oxidation, and backbone cleavage, have been well characterized [41,42]; however, recent evidence has revealed a novel class of chemical reactivity in which reactive lipid species covalently modify nucleobases within the mRNA, leading to the formation of lipid-mRNA adducts and subsequent loss of translational activity [2].

2.1. Discovery and Analytical Characterization of Lipid mRNA Adducts

In a pivotal study funded by Moderna, reversed-phase ion pair high performance liquid chromatography (RP-IP HPLC) was employed to analyze mRNA integrity, alongside conventional methods like capillary electrophoresis (CE) and agarose/polyacrylamide gel electrophoresis [2]. RP-IP HPLC separates molecules based on hydrophobic interactions between the analyte and stationary

phase, facilitated by high salt concentrations that neutralize the negative charge of the phosphodiester backbone [2]. Alkylammonium salts further create hydrophobic interactions, allowing high resolution, size dependent separation of mRNA molecules while retaining sensitivity to hydrophobic modifications[2].

Application of RP-IP HPLC to mRNA extracted from LNP formulations revealed a late-eluting peak (LP) not observed in CE analysis[2]. The LP eluted at 19–21 minutes, distinct from the main mRNA peak (MP) at 10–16 minutes, and exhibited an ultraviolet absorption maximum of 260 nm, confirming its RNA adjacent identity. Modified gradient conditions resolved the LP into a heterogeneous fingerprint of distinct peaks. Importantly, LP levels increased over time, and their abundance was strongly influenced by storage temperature [2].

2.2. Temperature Dependence of Lipid mRNA Adduct Formation

To assess the impact of storage conditions on adduct formation, mRNA LNP formulations were incubated for up to three months at −20°C, 5°C, 25°C, and 40°C, with samples analyzed by RP-IP HPLC at one-month intervals [2]. Across all temperatures, LP levels increased over time, with the most pronounced accumulation observed at 40°C, followed by 25°C, 5°C, and the lowest levels at −20°C [2]. These findings indicate that formation of lipid mRNA adducts is a thermally sensitive process, with elevated temperatures accelerating the covalent modification of nucleobases by reactive lipid species [2].

2.3. Isolation and Structural Characterization of Modified mRNA

In the study, mRNA extracted from LNPs was fractionated by RP-IP HPLC to generate purified MP and LP fractions [2]. Reanalysis confirmed that LP and MP fractions maintained distinct retention times in RP-IP HPLC, whereas CE and size-exclusion chromatography (SEC) showed no differences, ruling out aggregation or size-based artifacts [2]. SEC further confirmed that both MP and LP were predominantly monomeric, implicating increased hydrophobicity, rather than tertiary structure, as the source of LP retention [2].

Compositional analysis using UV spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), next-generation sequencing, and RNA oligonucleotide mapping revealed no changes in nucleotide sequence between MP and LP [2]. However, nucleoside profiling through enzymatic digestion followed by positive-mode LC-MS/MS identified several low-abundance (<1%) lipid-modified nucleosides exclusively in LP fractions. These modifications occurred across all four nucleobases (adenosine, cytidine, guanosine, and N1-methylpseudouridine), with fragmentation analysis confirming covalent addition of lipid chains to the nucleobase while leaving ribose unmodified [2].

2.4. Reaction Modeling and Identification of Reactive Lipid Components

To identify the source of adduct formation, binary reactions of mRNA with individual LNP components, including ionizable cationic lipid, polyethylene glycol (PEG) lipid, sterol, and phosphocholine were evaluated. LP formation occurred exclusively in the presence of the ionizable lipid, indicating that reactive species derived from this component drive covalent modification [2]. Binary reactions reproduced the RP-IP HPLC LP profile observed in full LNP formulations, demonstrating that these reactions can occur independently of the complete nanoparticle structure [2].

Further investigation across multiple ionizable lipid lots revealed variability in LP abundance, suggesting that minor oxidative impurities in the lipid component contribute to adduct formation. Time-course experiments demonstrated progressive LP accumulation over 1, 2, and 7 days, consistent with sequential addition of lipid molecules to mRNA [2]. Additionally, experiments using mRNA molecules of varying lengths (659–2498 nucleotides) indicated that longer mRNA sequences were associated with higher LP levels, reflecting the stochastic nature of adduct formation at the single-

nucleotide level [2]. Interestingly, while the total amount of adduct increased with mRNA length, the average retention time of individual LP species decreased, suggesting that the relative hydrophobic contribution of a single adduct is less pronounced in longer molecules [2].

2.5. Unrealized Implications

The researchers in the study were primarily focused on, in the journal article itself, the loss of RNA translation and why [2]. They did not expand into questions if reactive species is present in the COVID vaccines, that are creating covalent adducts within the vials of the vaccines, at the highest rate being closer to human body temperature in vitro–what this might theoretically mean within the human body if a reactive species is introduced, either by itself (as a free floating lipid), if it were attached to a piece of modified RNA entering a cell, and if the adducts, which are still potentially reactive, are attached to what has been found to be billions of pieces of linearized DNA plasmid existing inside of the LNP that may have also experienced covalent bonds, creating potential adducts. If GTI protocol states that the threshold is typically set at 1.5 µg/day, and if the researchers who tested and found adduct formation in the product itself, by molar mass and 1.5 µg/day limits that were established [4] without consideration for drugs with reactive species inside lipid formulations, tests and calculations must be done to rule in or out these serious concerns.

3. Mechanistic Deep Dive of Temperature and Charge

The formation of lipid-mRNA adducts within lipid nanoparticle formulations is profoundly influenced by both temperature and the charge state of the ionizable lipid components [2]. According to the Arrhenius principle, the rate of chemical reactions increases exponentially with temperature [42]. In the context of mRNA LNP systems, this foundation manifests as a marked increase of lipid nucleobase reactivity at elevated storage temperatures [2]. Experimental data from the Moderna study demonstrates that the relative abundance of the late-eluting peak, representing lipid modified mRNA species, increased progressively with temperature. mRNA LNP formulations stored at forty degrees Celsius over a three-month period exhibited significantly higher levels of late-eluting species compared to those stored at twenty-five degrees, five degrees, or minus twenty degrees Celsius [2]. This trend indicates that higher thermal energy accelerates the formation of covalent adducts between reactive lipid species and nucleobases, thereby directly reducing the proportion of unmodified, translationally competent mRNA [2].

In addition to thermal effects, the physical state of the lipid components also modulates reaction kinetics. Elevated temperatures increase lipid fluidity, enhance molecular mobility and encounter frequency between ionizable lipids and nucleobases [2]. The Moderna study's chromatographic analyses suggest that these increased encounters directly contribute to the observed accumulation of adducted mRNA specie [2]. Temperature dependent fluidity not only facilitates initial adduct formation but also allows for multiple sequential modifications along a single mRNA molecule, leading to broader heterogeneity in retention times and the characteristic polydisperse fingerprint of the late-eluting peak [2]

Electrostatic interactions further govern the propensity for adduct formation [6]. Protonation of the ionizable cationic lipid at physiological and acidic pH increases positive charge density and might enhance local proximity to the negatively charged mRNA phosphate backbone. This elevated local concentration of reactive lipid species in close contact with nucleobases facilitates covalent addition and thereby theoretically increases adduct yield. Interestingly, while high salt concentrations are traditionally used to screen the mRNA phosphate charges and allow retention based on hydrophobic interactions during reversed-phase ion pair high-performance liquid chromatography, ionic screening in the formulation environment may potentially, and paradoxically, increase local encounters. By partially neutralizing long-range electrostatic repulsion, screening allows lipids to approach nucleobases more effectively, which may promote the formation of additional adducts per mRNA molecule.

4. Hypothetical In Vivo Implications

A central question raised by the observations of Packer and colleagues is whether the electrophilic lipid derived species identified in LNP formulations [2] that are species capable of forming covalent adducts with RNA nucleobases in vitro, might under physiological conditions engage in similar or broader covalent chemistry with biomolecules inside the human body. The Moderna study [2] restricted its scope to adduction of RNA within the LNP itself, but the principles of organic chemistry, combined with the extensive toxicological literature on aldehyde and epoxide mediated biomolecular damage, argue for a wider consideration of possible, hypothetical downstream interactions in vivo [43]. The following analysis develops this mechanistic plausibility, distinguishing between what has been experimentally demonstrated and what remains hypothetical, while mapping established chemical pathways onto plausible biological encounter scenarios.

4.1. Chemical Reactivity and Thermal Acceleration

The chemistry underlying these adduction events of ionizable lipids are intrinsically electrophilic. Packer et al. confirmed that adduction events occur within the LNP, altering RNA chromatographic properties and abolishing protein translation [2]. Importantly, these reactions are kinetically favored at physiological temperature [2]. According to the Arrhenius principle, even modest increases in temperature from cold storage to 37 °C exponentially accelerate the reaction rates [2]. Moreover, at body temperature lipid bilayers become more fluid, increasing the diffusional mobility and local encounter frequency between electrophilic species and encapsulated nucleic acids [44]. Thus, both kinetic and thermodynamic factors argue that covalent chemistry observed in vitro could hypothetically proceed more efficiently in vivo.

4.2. Hypothetical Encounter Scenarios Inside the Body

The first and most direct target is the therapeutic mRNA itself. If electrophilic impurities remain associated with LNPs after administration, they may continue to react with the mRNA during circulation, endosomal escape, or after cytosolic release. The biological consequence of translational silencing of modified transcripts has been directly validated in vitro [2], and the extension to the intracellular environment is plausibly and chemically straightforward.

A second plausible target for these adducts to form is the impurities in the positively charged lipids to bind to the residual biotech plasmid DNA fragments, which have been detected in COVID vaccine lots at unexpectedly high copy number. The same nucleophilic sites susceptible to RNA are present in DNA, and DNA adducts are extensively documented in mutagenesis research [45]. Mutagenic events may not require plasmid integration into the genome; error prone repair of adducted DNA fragments present in the nuclear milieu might hypothetically be sufficient to generate mutations if such fragments gain access to the chromosomal environment. This interaction would occur if the fragments of linearized DNA plasmid inside of the LNP interacted electrostatically with the ionized cationic lipid particles which is a scientifically plausible mechanism, and form a similar covalent bond, resulting in adduct formation. This lipid DNA plasmid adduct, if imported into the nucleus of a cell, might be able to interact with the nucleic acids housed there. The positive charge on the ionizable lipid may assist in facilitating additional ease for nuclear import. During mitosis, nuclear envelope breakdown creates a transient opportunity for exogenous nucleic acids to contact chromatin. Protein mediated nuclear import pathways could carry nucleic acids complexed with host factors bearing nuclear localization signals [46]. Lipid nucleic acid complexes might destabilize membranes or exploit import machinery. The trafficking routes are chemically and biologically feasible, particularly in proliferating cells.

Proteins throughout the cytosol and extracellular space provide abundant nucleophilic sites [48]. Aldehyde and epoxide attack on cysteine thiols, lysine ε-amines, or histidine imidazoles is well documented in toxicology literature [49]. Within the context of LNP administration, such adduction could hypothetically lead to enzymatic inactivation, formation of misfolded proteins that perturb

proteostasis, or generation of covalently modified neoepitopes that engage adaptive immunity. In this way, proteins may serve both as sinks that quench reactive impurities and as functional casualties of chemistry.

4.3. Kinetic and Compartmental Constraints

The biological relevance of these encounter scenarios depends on the interplay of kinetics, concentration, and compartmentalization. Within the LNP, electrophiles are concentrated in proximity to nucleic acids, maximizing the probability of reaction [50]. Once released into plasma or cytosol, however, electrophiles encounter abundant nucleophiles like glutathione, albumin, and free amino acids, which act as scavengers and rapidly quench reactivity [51]. Physiological temperature enhances reaction rates [2] but also accelerates detoxification pathways. Thus, the temporal window for nucleic acid adduction hypothetically may be short, followed by dissipation of reactive species into relatively inert protein or thiol conjugates [52]. The balance between early nucleic acid targeting is a critical determinant of whether adduction events reach biologically significant levels.

4.4. Potential Cellular Outcomes

If such adduction events occur at sufficient frequency [2] the cellular consequences could, theoretically if conditions are right, include mutagenesis from DNA lesions, activation of DNA damage signaling pathways like p53, mitochondrial dysfunction with secondary reactive oxygen species production, and epigenetic perturbation from chromatin associated lesions. Protein adduction could generate immunogenic neoepitopes or disrupt enzymatic activities. Modified nucleic acids may engage innate sensors like cGAS–STING, further engaging APOBEC pathways [53], and provoking inflammatory cascades. These outcomes are hypothetical but grounded in established chemistry and cell biology.

4.5. Caveats and the Need for Empirical Testing

To date, no clinical evidence directly links lipid derived electrophile adduction to mutagenesis or pathology in vaccinated humans. Multiple protective barriers like dilution upon systemic distribution, rapid quenching by thiols, membrane trafficking limitations, and robust DNA repair reduce the probability that this specific chemistry manifests as disease [54]. However, the magnitude of risk scales with impurity burden [36], which is lot dependent and may vary between manufacturing runs. The presence of both reactive lipid species and residual plasmid DNA raises a non-zero, testable concern. Empirical work is required: kinetic competition assays between nucleic acids and physiological thiols, in vitro translation studies with deliberately adducted RNA, nuclear entry assays for adducted DNA, mapping of mitochondrial lesions, and mutagenesis screens in animal models. Only through such targeted studies can the field determine whether the chemical potential observed in vitro translates into biologically relevant outcomes in vivo.

5. Testing Needs

The findings of Packer et al. highlight a new dimension of chemical risk in lipid nanoparticle–based medicines [2]: the potential for covalent adducts between reactive lipid-derived species and nucleic acids. While the study provided the first systematic evidence of RNA adduct formation in vitro [2], it also underscored the absence of a standardized framework for evaluating these modifications under conditions relevant to human administration. Bridging this gap requires a coordinated program of experimental work spanning in vitro, in vivo, and human biomonitoring studies, supported by rigorous lot-to-lot and manufacturer-to-manufacturer comparisons.

The most immediate priority is in vitro testing under physiologically relevant conditions. The assays in Packer et al. were performed under controlled laboratory settings, but the chemical environment in vivo is more complex, involving body temperature (37 °C), a milieu of blood ions, and variable levels of reactive oxygen species generated during innate immune responses.

Reproducing these parameters in adductomic experiments is essential to determine whether the kinetics of adduct formation observed in the laboratory translate into the biological context. Such testing would also clarify the extent to which endogenous oxidative stress amplifies the production of electrophilic lipid species, thereby increasing adduction frequency.

In vivo animal models represent the next tier of inquiry. While it has been shown that in vitro assays can map chemical plausibility [2], only animal studies can establish whether RNA, DNA, or protein adducts accumulate in tissues following administration of LNP encapsulated nucleic acids. Sensitive mass spectrometry and sequencing based adductomics could be applied to harvested organs, particularly liver, spleen, lymph nodes, bone marrow, and other areas, where nanoparticles are known to accumulate [55,56]. These studies would further clarify whether reactive lipid impurities persist long enough in circulation to encounter host nucleic acids, whether they are metabolized or detoxified before such encounters, and whether downstream consequences like nuclear DNA or mitochondrial perturbation are engaged. Proteomics is also a concern, as these adducts may interact with any nucleic acids, like chaperones, motor proteins, and others.

Human biomonitoring studies represent the most critical step in evaluating clinical relevance. Non-invasive sampling strategies like liquid biopsies--encompassing plasma, urine, and cell-free nucleic acids (cfRNA, cfDNA) could provide evidence for covalent adducts in vaccine recipients [57]. Analogous assays are already used in chemical carcinogenesis research, where urinary excretion of DNA adducts serves as a biomarker of exposure to electrophilic toxins [58]. Applying these technologies to recipients of mRNA-LNP medicines would not only establish real world exposure profiles but also inform inter-individual variability in adduct burden, possibly linked to genetic background, age, or metabolic state.

A further dimension is comparative lot testing. Packer et al. reported inter-lot variability in adduct burden, correlated with impurity profiles in the lipid components [2]. Systematic analysis of different lots, storage conditions, and transport histories across manufacturers would clarify the extent to which adduction is a universal feature of the LNP platform or a contingent outcome of manufacturing quality. This would also help define best practices for minimizing reactive impurities through improved synthetic chemistry, purification, and cold-chain logistics.

Finally, it is essential to establish thresholds for biological significance. The Moderna study reported that fewer than 1% of nucleotides were covalently modified, but the impact of such a level remains unknown [2]. For DNA, even single adducts in critical regions can trigger repair responses or mutagenesis, while for RNA, adducts at key codons can abolish translation or alter protein fidelity [59–61]. A key research question is therefore what fraction of adducted nucleotides translate into measurable changes in gene expression, protein function, or cellular stress responses. Determining this threshold would provide the basis for regulatory standards analogous to those already applied to genotoxic impurities in small-molecule pharmaceuticals.

Comprehensive testing is required to move the issue of LNP-derived adducts from chemical plausibility to biological certainty. Only through integrated in vitro, in vivo, and human biomonitoring approaches, paired with systematic lot comparisons and threshold analyses, can the field determine whether the low-level covalent chemistry observed in Packer et al. carries real implications for safety and efficacy in the clinical setting.

Discussion

The Moderna study provided a landmark finding in the field of RNA based therapeutics: the clear demonstration that lipid derived electrophilic species can covalently modify nucleobases within therapeutic mRNA [2]. This result was not speculative but empirical, supported by chromatographic and mass spectrometric evidence, and accompanied by the functional consequence of translational silencing [2]. In so doing, it established beyond doubt that covalent adduct formation is a chemical reality within lipid nanoparticle formulations [2]. The remaining question is no longer whether such reactions can occur, but whether they extend in vivo to biologically meaningful outcomes. Mechanistic extrapolation suggests several pathways by which the chemistry documented in vitro

could be amplified within the physiological environment [2]. First, the increase in temperature from cold storage to body heat accelerates the kinetics of electrophile–nucleophile reactions, while lipid fluidity at 37 °C increases the local encounter rate of reactive impurities with encapsulated nucleic acids. Second, the in vivo milieu is not chemically inert: reactive oxygen species generated during immune activation may oxidize lipids further, producing secondary electrophiles that extend the lifetime and diversity of reactive species [62]. These considerations raise the possibility that the modest adduct levels observed under laboratory conditions may underrepresent what occurs in situ.

The presence of plasmid DNA contaminants in some vaccine lots, as documented by independent and other laboratories [36], adds another dimension of plausibility. DNA contains chemically similar nucleophilic sites as RNA and could also be a canonical substrate for chemical mutagenesis. Should lipid derived electrophiles react with linearized plasmid DNA fragments which have been found by researchers as contamination in COVID vaccines [36], adducted DNA fragments might persist in the cytosol, interact with host repair machinery, and under certain circumstances gain access to the nucleus. While integration is not required for mutagenic effects--miscoding and error-prone repair suffice--the nuclear entry of modified DNA raises a nontrivial question of genotoxic liability that has not been systematically evaluated [63,64].

Beyond nucleic acids, proteins present an abundant and chemically attractive target for covalent bonds and adduct formation. Covalent modification of proteins by electrophiles is a well-established mechanism of toxicity in multiple chemical exposures, from industrial aldehydes to endogenous lipid peroxidation products [65]. Protein adduction can inactivate enzymes, distort signaling cascades, or create neoantigens that alter immune recognition [66]. Yet the “protein adductome” of LNP-derived impurities remains almost completely unstudied, representing a significant blind spot. In the context of regulatory toxicology, this omission is striking, given that small-molecule drugs with comparable genotoxic impurity risks are subject to stringent thresholds and extensive adduct testing.

The parallel to the regulatory framework for GTIs in traditional pharmaceuticals is unavoidable. For small molecules, even trace-level GTIs trigger strict regulatory limits and require orthogonal analytical assays to ensure control. The empirical finding that lipid nanoparticles can harbor electrophilic species capable of covalent adduction suggests that a similar standard may ultimately be necessary for RNA-based medicines. Testing using high-resolution mass spectrometry to detect RNA, DNA, or protein adducts should become a required release assay, complementing existing purity and potency metrics. Such a shift would bring the LNP platform into alignment with established toxicological principles rather than leaving it in a regulatory vacuum.

At stake is not only scientific rigor but also ethical obligation. Patients and the public deserve transparency about the presence of impurities capable of covalent reactivity, particularly when those impurities may alter the pharmacologic function of the therapeutic nucleic acid or pose theoretical risks of genotoxicity and immunogenicity. Acknowledging the existence of adduct formation, quantifying its extent, and testing for its downstream consequences should be viewed not as a threat to the RNA therapeutic platform but as a maturation of its quality framework. By proactively addressing these questions, developers and regulators can safeguard both public trust and scientific credibility.

The Moderna study confirmed the chemical reality of nucleic acid adduction by reactive lipid impurities [2] but left unanswered the biological scope of the phenomenon. Theoretical considerations argue for possible amplification in vivo through temperature, reactive oxygen species, and DNA contaminants. Protein adduction and genotoxic parallels remain largely unexplored. Together, these gaps underscore an urgent need for systematic research and regulatory adaptation. Transparency and comprehensive testing are not optional add-ons but ethical imperatives, ensuring that the remarkable promise of RNA therapeutics is realized without avoidable chemical liabilities.

This work does not discount other concerns studied by scientists from around the world researching the concerns of the COVID vaccine platform.

Conclusion

The findings reported in Moderna's impurity analysis, together with accumulating independent observations, make clear that RNA LNP platforms may carry adduct formation risks that cannot be dismissed as mere manufacturing artifacts. The observation of covalent lipid–RNA adducts, even at levels reported to be below one percent, represents only an initial glimpse into a broader and insufficiently characterized phenomenon. These early data should be treated not as reassurance, but as a baseline demanding rigorous and comprehensive inquiry.

Systematic investigations across adductomics, proteomics, and genotoxic impurity profiling are urgently required to define the full scope of reactivity within these formulations. Such studies must extend beyond the RNA cargo to encompass DNA contaminants, host proteins, and nuclear and mitochondrial interactions, all of which are biologically relevant targets for covalent modification. The absence of such data leaves a critical knowledge gap regarding potential long-term effects, including mutagenesis, protein dysfunction, and immunopathology. Before further expansion of the RNA LNP platform into vaccines, therapeutics, or gene editing applications, independent verification in human systems must be prioritized. Transparent release testing, cross-lot comparisons, and biomonitoring strategies are not optional but essential safeguards. Only through a deliberate and methodical approach to impurity and adduct characterization can the platform realize its therapeutic potential while ensuring public trust and biological safety.

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